Fig.6c: 293T cells were transiently transfected with the indicated plasmids. 24 hours post-transfection, cells were harvested and analyzed by flow cytometry. Apoptosis of GFP-positive cells was analyzed by Annexin-V/PI stain.
Fig. 6d: Western blot analysis of transiently transfected 293T cells 24 hours post-transfection, showing the expression of the appropriate proteins.
Abbreviations: Ap., apoptosis; N. treat, no treatment; treat., treatment; E. vec., empty vector; Ab., antibody.

Figure 7a-~~d~~c: Post-vaccination metastatic melanoma cell line over-expresses Livin, rendering the cells resistant to chemotherapy.
(a) Melanoma cell lines were lysed, normalized for total protein and analyzed for Livin, XIAP and Survivin expression.
(b) Melanoma cell lines LB33 Mel A1 and B1 were treated with etoposide (15 µg/ml). Apoptosis rate was determined by nuclear morphology, as described.
(c) Western blot analysis of the Melanoma cell line LB33 Mel A1 and B1 for Livin (upper panel) and PARP cleavage as a marker of apoptosis (lower panel)
Abbreviations: Ap., apoptosis; H. po.-treat., hours post-treatment; F.l., full-length.

Figure 8a-c: Livin expression in primary melanoma cells mediates etoposide resistance.
Fig. 8a: Livin, XIAP and Survivin expression was determined in 19 primary cultures of melanoma cells derived from patient's tumors (numbers indicate patient's code).
Fig. 8b: Six samples were selected according to the level of Livin expression (high: 5556, 55112, moderate: 5524, 55164, or undetectable: 5530, 5533). These samples were treated with etoposide (20 µg/ml). Apoptosis rate was determined by nuclear morphology, as described. The data shown are representative of three independent experiments, which were also confirmed by flow cytometry, using sub-G1 assay.